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ROY WALDO MINER

IONIC TRANSFER IN NERVE IN RELATION TO BIO-ELECTRICAL PHENOMENA

BY

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IONIC TRANSFER IN NERVE IN RELATION TO BIO-ELECTRICAL PHENOMENA*

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† With the technical assistance of Mrs. Elizabeth Bowman.

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IONIC TRANSFER IN NERVE IN RELATION TO BIO-ELECTRICAL PHENOMENA

Introduction

The electrical potential difference between the interior and exterior of living cells, referred to as resting potential, polarization, *etc*., is probably a universal biological property. Its study has proven simplest in nerve fibers, where its production and utilization serve as the major and possibly sole functions. Inasmuch as modifications in the electrical state of nerve are intimately associated with alterations in other nerve phenomena (*e.g*., see Bishop, 1932, Lorente de Nó, 1947), an insight into their cause appears essential for an adequate understanding of other aspects of nerve functioning.

Recent developments, chiefly from English laboratories (Keynes, 1949; 1951a,b; Keynes and Lewis, 1951a,b; Hodgkin, 1949; Hodgkin *et al*., 1949; Hodgkin and Katz, 1949; Rothenberg, 1950), provide evidence for a relation between highly transient electrical changes, and sodium and potassium movement. As recognized by these investigators, however, the data are either extremely indirect and dependent on a number of assumptions or seriously dissociated in time from the bio-electrical events assumed to be involved.

Our own earlier studies (*e.g*., Shanes, 1948a,b; 1949a,b; Shanes and Hopkins, 1948) led to the conclusion on indirect grounds, despite some evidence to the contrary (Cowan, 1934; Fenn, 1934), that slow polarization changes in both vertebrate and invertebrate nerve could be accounted for by the movement of potassium out of, or back into, the fibers. It was noted that the electrical fluctuations were explainable, at least initially, in terms of potassium concentration changes at the surface of the fibers, rather than in the interior, where the normal high content of this ion would require a far greater transfer to achieve the concentration changes needed to account for the magnitude of the electrical effects. The predicted potassium shifts for anoxia have recently been confirmed (Fenn and Gerschman, 1950; Feng *et al*., 1950a; Shanes, 1950a).

The order of time involved in the slow bio-electrical phenomena gave promise of permitting the detection of associated ion transfer, if such should occur. The present report describes the potassium and sodium shifts which have actually been found in frog sciatic nerve under conditions similar to those of the earlier bio-electrical experiments. Additional data will be presented which appear to distinguish the specific ion directly concerned with the electrical changes. These new results justify the development of equations for the kinetics of potassium movement under certain conditions. Preiminary reports have appeared on certain aspects of this work (Shanes, 1950c, 1951c). Still more data, acquired to test further the theoretical treatment, will also be presented. Some of the implications of the present study

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[†] The capable assistance of Miss Tess Abramsky in exploratory studies is gratefully cknowledged.

for the more transient bio-electrical properties of nerve will be discussed briefly.

Methods

All studies were carried out on Rana pipiens sciatic nerves dissected from the point of emergence from the vertebral column to the knee. Two paired sets of nerves, each set composed of two nerves tied together at their ends, contributed to a single day's run.

The apparatus employed was described previously (Shanes, 1950a, 1951a). To summarize, four specially designed pyrex circulation units, two of the type described in the earlier paper and two in the later one, were used in conjunction with two pyrex reservoir units with continuous oxygen or nitrogen flow to permit (a) recirculation or agitation of small volumes of solution (*ca.* 0.7 ml.) in contact with individual sets of nerves; (b) removal of these samples under oxygen or nitrogen at regular intervals, usually for potassium analysis; and (c) their replacement with fresh solution in the continued presence or absence of oxygen.

Most short experiments, lasting six hours or less, were carried out with freshly dissected material. Each set of nerves was dried by contact with filter paper and quickly weighed on a torsion balance before and after each run. Samples were collected at hourly or two-hour intervals. At the termination of the experiment, the ends, with the short lengths of thread tied to them, were cut off and the rest of the tissue was dried to constant weight. It was then incinerated for 15 hours at 500°C. The ash was taken up in water over a four-hour period for direct analysis for both sodium and potassium Acidification was found unnecessary. Thus, two distinct paired experiments were carried out at one time. The pairing procedure increased the reliability of comparisons. This is evident from the smaller standard error of differences computed from the differences of pairs than from the standard errors of the means. For this reason, only the former is given for differences in the tables. A second check on the significance of differences with respect to potassium was provided by the availability of data from both media and nerves. Sodium and potassium determinations were carried out on identical tissue samples, to which may be attributed the high level of significance of differences established for the transport of these ions.

All analyses were performed with the Beckman flame spectrophotometer. The usual precautions were taken to make up standards with concentrations of sodium and potassium, and other constituents as similar to that of the unknowns as possible. The average deviation of readings was of the order of ½ per cent. The internal agreement of the data, as well as the agreement with the literature where comparable figures are available (*e.g.*, Fenn *et al.*, 1934), indicate that the methods were quite satisfactory.

(*e.g.*, Fenn *et al.*, 1934), indicate that the methods were quite satisfactory.

Experiments of long duration, in which ionic shifts were followed for 24 hours, employed nerves that had been equilibrated for 16 hours in oxygenated Ringer at 4–5°C. Low equilibration temperatures were used routinely, although the potassium and sodium content of nerves kept at room temperature for similar periods of time are no different. The sodium and potassium evels after these long equilibration times were only slightly different from hose of fresh preparations (*cf.* TABLES 1–3 with TABLES 7 and 8); all 60 reparations so treated gained weight, the average being about seven per ent.

At the end of the equilibration period, the nerves were placed in experimental solutions at room temperature for a half hour, then mounted in he units with either oxygen or nitrogen flowing, depending on whether erobic or anaerobic conditions were desired. Within one hour after removal of the nerves from the cold room, the experiment was begun by iscarding the experimental solutions that had been in contact with them nd adding fresh oxygenated or oxygen-free solutions. Thus, 15 to 30 minutes of nitrogen flow assured establishment of anoxia, and a full hour was allowed for penetration of experimental agents before solutions to be sed for meas-urement were applied. Samples in these experiments were ollected at three-hour intervals for the first 12 hours. A single collection was made 12 hours later, the volume of medium in contact with the nerves uring this period being two to three times that of the short collections.

All solutions were made isotonic with 0.111 M NaCl. The Ringer conained the usual electrolytes in the following concentrations: 107 mM NaCl, 1.7 mM KCl, 1.1 mM $CaCl_2$, and a pH 7.2 all-sodium Sørensen phosphate buffer osmotically equivalent to 1 mM NaCl. All additions involving smotically effective quantities were made by removal of an equivalent mount of sodium; the pH of experimental solutions was adjusted to the ame level as Ringer. Room temperatures at which the experiments were erformed were usually within the range of 24–28°C. The temperature ariation during the course of most experiments was less than 2°.

Results of Short-term Experiments

Anoxic Effects. TABLES 1 and 2 summarize data which have been obained. Analyses for potassium, carried out on the media, are in general greement with the results obtained for the nerves themselves. They emonstrate a liberation of potassium in the absence of oxygen, the amount eing greater at longer times (*cf.* five- and six-hour losses with two-hour mounts) and in the presence of veratrine at concentrations ranging from .17 to 1.0 mg. per cent. In the case of the alkaloid mixture, a somewhat igher potassium loss was noted in the preliminary baseline period in O_2, articularly at the higher concentrations; this amounted to 1.2 μ M/g. in wo hours as compared to 0.18 for the controls of the same series. These osses in oxygen were deducted from the nitrogen data to give the corrected alues in TABLE 1, which still show a significantly larger potassium loss in he veratrine.

The same tables demonstrate that potassium liberation during anoxia reduced by low concentrations of cocaine and glucose. The controls in he case of the sugar contained an osmotically equivalent amount of sucrose. he glucose effect is completely blocked by 1 mM iodoacetate.

Analyses of the nerves supplied data concerning the behavior of sodium. he same tables show that potassium loss, or modifications in the loss, are ccompanied by a consistently greater sodium uptake or alteration in upke.

The nerve weights prior to and following the experimental procedures provide an index of water movement. In TABLE 1, a consistent and significant water absorption occurs in nitrogen as compared to the controls, in agreement with the findings of Fenn and Gerschman (1950). In these, and especially in most subsequent experiments, water movement was so small that it had a negligible effect on data even if the latter are referred to final rather than initial wet weights. As a further check on a possible influence of water uptake, most results are expressed relative to dry, as well as wet weights (*cf.* first series in TABLE 2).

TABLE 1

COMPARISON OF THE SODIUM AND POTASSIUM CONTENTS OF PAIRED NERVES UNDER DIFFERENT EXPERIMENTAL (X) AND CONTROL (C) CONDITIONS AND CORRESPONDING POTASSIUM DATA FROM MEDIA ANALYSES†

Conditions	*No. of pairs*	*Nerve data: collected after 6 hr. exposure*						*Medium data:* K	
		μM/g. initial wet weight				*Weight change*		*5 hr. loss/g. wet wt.*	
		Na		K		X	C	X	C
		X	C	X	C				
X = N_2 C = O_2 10/5–10/12/50	6	82.0	66.3	27.1	38.4	2.7	−4.7	11.4	1.7
	σ_M	±2.3	±1.7	±1.3	±1.2	±1.7	±1.3	±1.2	±0.2
	$\Delta = X - C$	15.7		11.3		7.4		9.7	
	$\sigma_{\overline{\Delta}}$	±1.5		±1.3		±1.5		±1.2	
	$\Delta Na - \Delta K$	4.4							
	$\sigma_{\overline{\Delta\Delta}}$	±2.3							
								6 hr. loss	
X = N_2 C = O_2 1/2–1/7/50	5					3.8	−1.2	6.6	1.0
	σ_M					±1.3	±0.2	±0.1	±0.2
	Δ					5.0		5.6	
	$\sigma_{\overline{\Delta}}$					±1.3		±0.5	
								5 hr. loss	
X = N_2 + 0.1% Coc. C = N_2 10/17–10/18/50	4	69.9	77.7	35.0	29.8	1.7	4.8	9.0	12.2
	σ_M	±1.6	±2.3	±1.8	±1.5	±1.7	±0.5	±1.0	±0.6
	Δ	7.8		5.2		3.1		3.2	
	$\sigma_{\overline{\Delta}}$	±2.1		±0.5		±1.5		±0.6	
								2 hr. loss	
X = N_2 + 0.33 mg% Ver* C = N_2 2/28–3/6/50 4/10–4/14/50	5							5.4	2.0
	σ_M							±1.0	±0.18
	Δ							3.4	
	$\sigma_{\overline{\Delta}}$							±0.84	

* Mean veratrine concentration, with range from 1 to 0.17 mg. %.

† Standard errors of the mean (σ_M) of the difference of paired nerves ($\sigma_{\overline{\Delta}}$), and of the difference in the changes in sodium and potassium ($\sigma_{\overline{\Delta\Delta}}$) are given as measures of variability in this and following tables. Coc. = cocaine, Ver. = veratrine.

Potassium liberation and its alterations are therefore in the proper direction to account for: (a) depolarization during anoxia, (Gerard, 1930, Shanes and Brown, 1942); (b) reduced anoxic depolarization in the presence of glucose (Shanes, 1948 b, 1949 b) and cocaine (Shanes, 1948a, 1951b); (c) augmented anoxic depolarization in veratrine (Shanes, 1948c, 1951b); and (d) counteraction of the glucose effect by iodoacetate (Shanes, 1951b).

Moreover, these results demonstrate, also for the first time, that potassium movement in nerve under these conditions is largely an exchange for extracellular sodium.

Veratrine Action. In addition to the enhancement of the anoxic depolarization at low concentrations, the alkaloid mixture can itself cause a progressive depolarization at higher concentrations (Lorente de Nó, 1947) and this effect can be completely blocked with 0.1 per cent cocaine or less (Shanes, 1951b). The effect on potassium and sodium distribution was therefore examined under similar conditions.

TABLE 2

THE EFFECT OF ANOXIA, IN THE PRESENCE OF DIFFERENT EXPERIMENTAL (X) AND CONTROL (C) SOLUTIONS, ON POTASSIUM LIBERATION AS ESTIMATED FROM MEDIA ANALYSES, AND ON THE SODIUM AND POTASSIUM CONTENTS OF PAIRED NERVES*

Conditions	No. of pairs	*Nerve data: collected after 6 hr. exposure*										*Medium data: 5 hrs.*	
		μM/g. dry weight				*μM/g. wet weight*				*Wt. change per cent*		*μM K loss/g. wet wt.*	
		Na		K		Na		K					
		X	C	X	C	X	C	X	C	X	C	X	C
X = 10 mM Gluc. in R.	6	265	310	129	114	58.7	67.9	28.7	25.5	3.9	2.2	7.0	10.8
C = 10 mM Suc. in R.	σM	±14	±14	±18	±16	±1.8	±2.0	±1.1	±0.8	±1.0	±0.9	±0.5	±0.5
12/4–12/6/50	Δ = X − C.......	45		15		9.2		3.2		1.7		3.8	
	σΔ̄.......	±15		± 3.3		±3.3		±0.7		±0.9		±0.2	
												2 hr. loss	
X = 10–20 mM Gluc. in R.	6									−1.9	−1.7	0.67 ±0.2	2.8 ±0.7
C = 10–20 mM Suc. in R.	σM												
5/8–5/10/50 /28–10/9/50	Δ.........											2.1	
	σΔ̄.........											±0.6	
X = 1 mM IAA + 10 mM Gluc.	4									−3.1	−1.9	2.71	0.74
C = 10 mM Gluc.	σM									±1.2	±0.9	±0.3	±0.4
1/24–1/26/50 5/12–5/16/50	Δ									−1.2		1.97	
	σΔ̄.........									±1.3		±0.13	

* Gluc. = glucose, Suc. = sucrose, IAA = iodoacetate.

FIGURE 1(A) illustrates the time course of potassium liberation when veratrine is applied to Ringer nerve; it is apparent that the presence of 0.1 per cent cocaine completely prevents potassium escape. Figure 1(B), the average of four paired experiments, each of which gave essentially the same results, shows a gradual falling off of potassium escape in veratrine; cocaine not only stops the loss of potassium immediately but consistently leads to a reabsorption of the ion, despite the continued presence of veratrine.

TABLE 3 summarizes additional data obtained from both media and nerve analyses and from dry and wet weights of the nerves. The figures are given in three groups because of significant differences observed in the relative shifts of sodium and potassium. In all groups it is apparent (a) that veratrine causes a release of potassium; (b) that this is accompanied by an uptake of sodium; (c) that cocaine prevents the ion interchange; and (d) that the weights and the ratios of dry to wet weight remain essentially

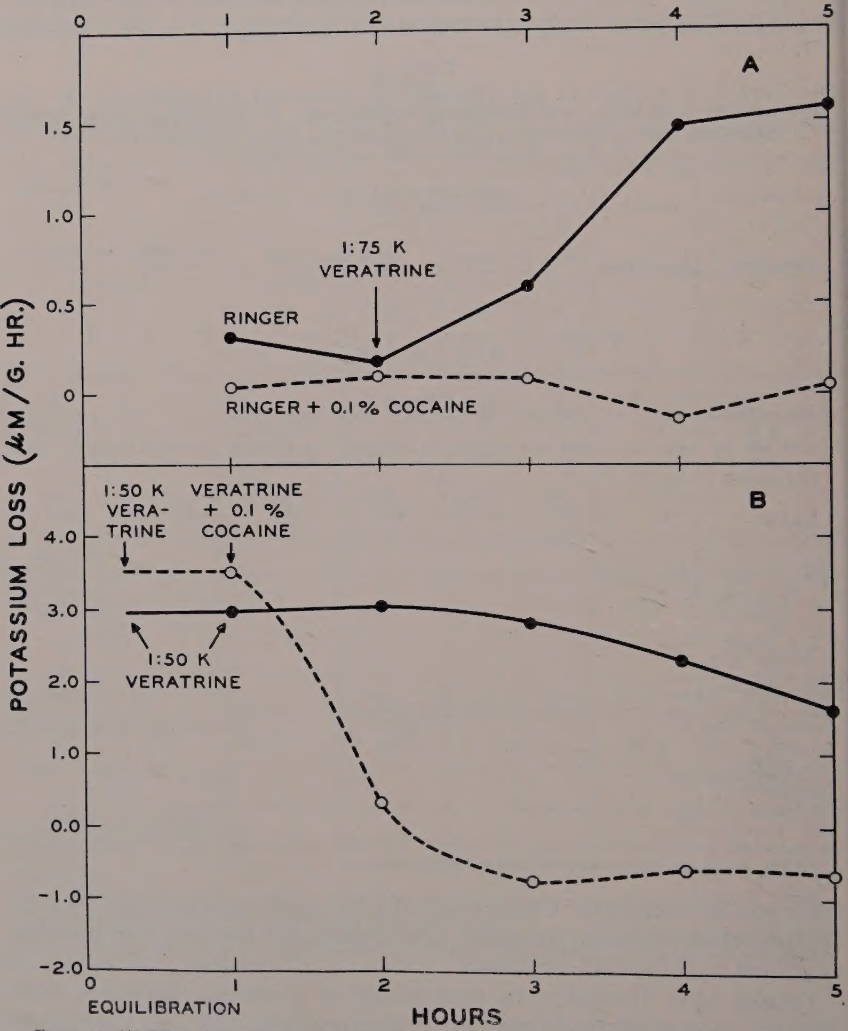

FIGURE 1. (A) Effect of veratrine on the hourly potassium release in the presence and absence of cocaine. (B) Rate of potassium release in veratrine compared with that following the addition of cocaine.

constant. The three groups differ in one important respect, *viz.*, the relative amounts of sodium and potassium transferred. Thus, in the first group, composed of nerves taken from animals stored several weeks in a frog tank, sodium entry is significantly less than the potassium released, whereas in the second group, done during the following week with freshly received frogs, the situation is reversed. The third group, in which the experimental

TABLE 3

COMPARISON OF THE SODIUM AND POTASSIUM CONTENTS OF PAIRED NERVES, AND CORRESPONDING POTASSIUM DATA FROM MEDIA ANALYSES, UNDER DIFFERENT EXPERIMENTAL (X) AND CONTROL (C) CONDITIONS*

Conditions	No. of pairs	*Nerve data: Collected after 6 hr. exposure*												*Medium data: μM K/g. wet wt.*			
		μM/g. *dry weight*				μM/g. *wet weight*				*Weight change*		$\frac{\textit{Dry weight}}{\textit{Wet weight}}$		*First 2.5 hr. loss*	*Second 2.5 hr. loss*	*Total 5.0 hr. loss*	
		Na		K		Na		K									
		X	C	X	C	X	C	X	C	X per cent	C per cent	X per cent	C per cent	X	X	X	C
X = V. in R.	7	345	308	105	179	74.4	66.6	22.6	38.8	2.47	0.64	21.6	21.9	5.7	5.8	11.5	0.8
C = R.	σ_M	±10	±9.5	±9.2	±1.6	±2.1	±1.2	±1.9	±0.5					±0.8	±0.5	±1.0	±0.2
11/6 – 11/9/05	$\Delta = X - C$	37		74		7.8		16.2		1.8		0.3				10.7	
	$\sigma_{\bar{\Delta}}$	±4.4		±8.3		±1.3		±2.0		±0.5		±0.18				±1.3	
	$\Delta Na - \Delta K$	37				8.4											
	$\sigma_{\overline{\Delta\Delta}}$	±8.5				±2.5											
X = V. in R.	7	382	275	92.1	163	84.4	62.5	20.6	37.3	0.25	0.24	22.6	23.2	7.2	5.5	12.7	0.06
C = V. + Coc. in R.	σ_M	±25	±16	±5.0	±4.2	±2.2	±1.9	±1.3	±0.5					±0.5	±0.3	±0.6	±0.3
11/13 – 11/16/50	$\Delta = X - C$	107		71		21.9		16.7		(0.01)		0.55				12.6	
	$\sigma_{\bar{\Delta}}$	±8.3		±6.6		±1.4		±0.9				±0.38				±0.5	
	$\Delta Na - \Delta K$	36				5.2											
	$\sigma_{\overline{\Delta\Delta}}$	±7.5				±0.9											
X = V. in R.	6	358	264	74.4	155	80.9	60.8	16.8	36.1			22.6	23.4	7.9	5.3	13.2	−0.35
C = V. + Coc. in R.	σ_M	±15	±14	±4.4	±9.9	±2.1	±2.2	±0.8	±1.8					±0.2	±0.3	±0.4	±0.29
11/27 – 11/29/50	$\Delta = X - C$	94.7		80.3		20.1		19.3				0.8				13.6	
	$\sigma_{\bar{\Delta}}$	±16		±6.6		±2.4		±1.2				±0.5				±0.4	
	$\Delta Na - \Delta K$	14.4				0.7											
	$\sigma_{\overline{\Delta\Delta}}$	±21															

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* V. = 2 mg. % veratrine, Coc. = 0.1% cocaine, R. = Ringer.

procedure is identical with that used in the second but in which the older batch of frogs was employed again, occupies an intermediate position, so that the sodium and potassium shifts are about equal. The procedures and temperatures of these experiments were identical, hence it appears that the fresh frogs differed in a manner, possibly seasonal, which was being approached by the animals kept in captivity. The potassium levels and the magnitudes of the shifts of this ion were essentially unchanged. The control levels of sodium, however, were significantly lower at later times. This effect is not attributable to the use of cocaine, for the same difference is also apparent from a comparison of the sodium content in X and C columns of the first rows in TABLES 2 and 1 respectively. These lower initial concentrations, together with a somewhat greater final concentration, contributed to the larger sodium uptake observed at later times. A tentative conclusion suggested by these results is that, although potassium loss is accompanied by sodium entry, the latter may be more readily depressed.

TABLE 4

THE HOURLY LOSS OF POTASSIUM (μM/g. WET WEIGHT) IN 5 mM IODOACETATE, MEASURED AT SUCCESSIVE HOURLY INTERVALS (C, C′), AND IN THE ADDITIONAL PRESENCE OF COCAINE (X)*

Hour	1		2			3		4		5	
	C	C′	C	C′		C	X	C	X	C	X
K lost	1.53	1.54	2.12	1.81	0.1% Coc. in C′ = X	2.43	1.77	2.70	1.70	2.62	1.94
σ_M	±0.10	±0.14	±0.26	±0.18		±0.28	±0.36	±0.18	±0.16	±0.22	±0.20
$\Delta = C - C' = C - X$	−0.01		0.31			0.66		1.00		0.68	
σ_Δ	±0.17		±0.27			±0.40		±0.13		±0.09	

* Eight paired experiments.

Additional evidence will be presented later. For the present, it suffices to note the correlation which has been established between potassium movement and bio-electrical changes in the presence of these representatives of "stabilizing" and "unstabilizing" drugs.

An important feature of cocaine action should also be noted: The anaesthetic is far more effective in counteracting the action of veratrine than that of metabolic inhibition as exemplified by anoxia. The weaker antagonism is apparent in another case of inhibition, *viz.*, iodoacetate poisoning. TABLE 4 gives the averaged data showing the potassium loss per hour in eight paired nerves during the action of 5 mM iodoacetate. This loss is in keeping with the depolarization caused by the glycolytic inhibitor (Shanes and Brown, 1942). The presence of 0.1 per cent cocaine in the third hour and thereafter in one of the pairs causes a 30 per cent decrease in potassium loss which becomes highly significant in the fourth and fifth hours. The magnitude of the protective action is about the same for anoxia (TABLE 1).

Effects of a Low Sodium Ringer. The predictability of potassium behavior is in favor of the original hypothesis that transfer of this ion underlies the bio-electrical changes observed under the same conditions. However, the new finding that the potassium shifts are achieved largely by exchange with sodium raises the question of which ion is more closely linked with metabolism and more intimately concerned with the electrical changes. This is particularly important in view of the recent tendency to emphasize the sodium ion in other systems (*e.g.*, Ussing, 1949a; Flynn and Maizels, 1950).

If, as some have maintained, the high intracellular potassium content is due to a sodium exclusion mechanism, it follows that potassium escape must be attributed to the failure of sodium extrusion. In other words, potassium loss should invariably be accompanied by a sodium uptake of at least equal magnitude. One exception has been noted in TABLE 3 (see also TABLE 8).

A more striking exception is shown in TABLE 5. In this experiment, one of each of eight pairs of nerves served as a control in Ringer, while the other was continually exposed to a 2 mM sodium Ringer containing all the other usual constituents and in which the sodium deficit was compensated with an osmotic equivalent of sucrose or choline chloride. After a one hour equilibration period, media samples were collected at two one-hourly intervals in oxygen, and then, after an additional half-hour equilibration in nitrogen, samples were collected at two two-hourly intervals. Only potassium increments were determined in the Ringer samples. The low sodium Ringer permitted analysis for both sodium and potassium. The results show (a) a continuing loss of potassium in oxygen in low sodium instead of the more usual decrease in loss in Ringer (the maintained loss in the presence of oxygen was found to continue for much longer exposures to low sodium*, and consequently longer equilibration periods proved impracticable); (b) a practically identical potassium loss during the first two-hour interval in nitrogen regardless of the sodium content of the medium, and a somewhat smaller loss in low sodium during the second nitrogen interval; and (c) a continually decreasing liberation of sodium from the nerves with no sudden transition on going from oxygen to nitrogen.

TABLE 5

POTASSIUM LOSSES TO RINGER, AND SODIUM AND POTASSIUM LOSSES TO A 2 mM SODIUM RINGER, MEASURED AT SUCCESSIVE INTERVALS, FIRST IN OXYGEN, THEN IN NITROGEN§

Conditions	*Ringer* K *loss*	*Low sodium Ringer**	
		K *loss*	Na *loss*
1 hr. O_2	0.24 ± 0.10	0.34 ± 0.15	3.88 ± 0.19
1 hr. O_2	0.08 ± 0.11	0.31 ± 0.13	2.49 ± 0.25
2 hr. N_2	2.02 ± 0.19	2.13 ± 0.20	1.91 ± 0.18†
2 hr. N_2	3.50 ± 0.15	2.58 ± 0.19	1.05 ± 0.24‡

* NaCl replaced with sucrose or choline chloride.
† The expected sodium loss calculated from oxygen data is 2.10 μM/g.
‡ The expected sodium loss calculated from oxygen data is 0.86 μM/g.
§ Data in μM/g. wet weight; eight paired experiments.

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* After 16 hours in low sodium, potassium losses in oxygen were still apparent. It is possible that the metabolic inhibition associated with low sodium levels (*cf.* Riley, 1950) may be responsible.

The release of sodium (and chloride) is chiefly from the extracellular space and approximates an exponential function (Fenn *et al.*, 1934). Preliminary measurements, given in FIGURE 2(A), substantiate the exponential time course of sodium diffusion. Losses over successive two-hour intervals are plotted. The mean time constant, calculated from these losses to the medium, is found to be 140 ± 45 minutes (for method see theoretical section). This is in reasonable agreement with the figure of 200 minutes estimated from the chloride diffusion shown in a figure by Fenn *et al.* (1934). Their data are replotted semilogarithmically in FIGURE 2(B). Thus, it has been possible to calculate the normal sodium loss to be expected in the nitrogen phase from the figures in oxygen, thereby permitting a more critical check of whether sodium is absorbed. These are given in TABLE 5. No significant difference exists, and it must be concluded, therefore, that no sodium uptake occurs at all in nitrogen at low sodium levels, despite a large loss of potassium. This fact, together with the small effect of a drastic lowering in the sodium in the medium on potassium outflux, demonstrate the independent nature of potassium movement under conditions of anoxia. Presumably, another ion can function in place of sodium to permit the exit of potassium, either another extracellular cation for exchange or an intracellular anion for diffusion with the potassium. The latter is the more likely situation since the nerves in low sodium lost a significant amount of weight (7.4 ± 2.1 per cent) in nitrogen whereas the Ringer controls did not. In any case, it is clear that the inward movement of sodium in normal Ringer during anoxia cannot be regarded as responsible for the outward migration of potassium.

The possibility of an effect by low sodium on potassium release by veratrine also was explored. The averaged data, obtained as in TABLE 5, are given in TABLE 6. A difference from the anoxia experiments is immediately apparent, *viz.*, the potassium appearing in veratrine is substantially reduced by the sodium deficit. This supplements the cocaine experiments in indicating an important difference in the mode of action of metabolic inhibition and of veratrine. Moreover, it suggests that cocaine and low sodium act in a similar manner. Again, no significant uptake of sodium is seen to occur when potassium release is augmented in the low sodium Ringer. No consistent weight changes were noted in either Ringer.

Results of Long-term Experiments

The conformance of potassium movement with that predicted from the many conditions under which bio-electrical changes had previously been observed appeared to justify a detailed analysis of the kinetics. A satisfactory mathematical description of potassium behavior could be expected to provide a more critical test of the postulated role of potassium in bioelectrical phenomena.

As the quantitative theory—presented in the next section—was developed, it became apparent that experiments carried out over 24 hours offered a better basis for comparison. Such prolonged experiments will now be described.

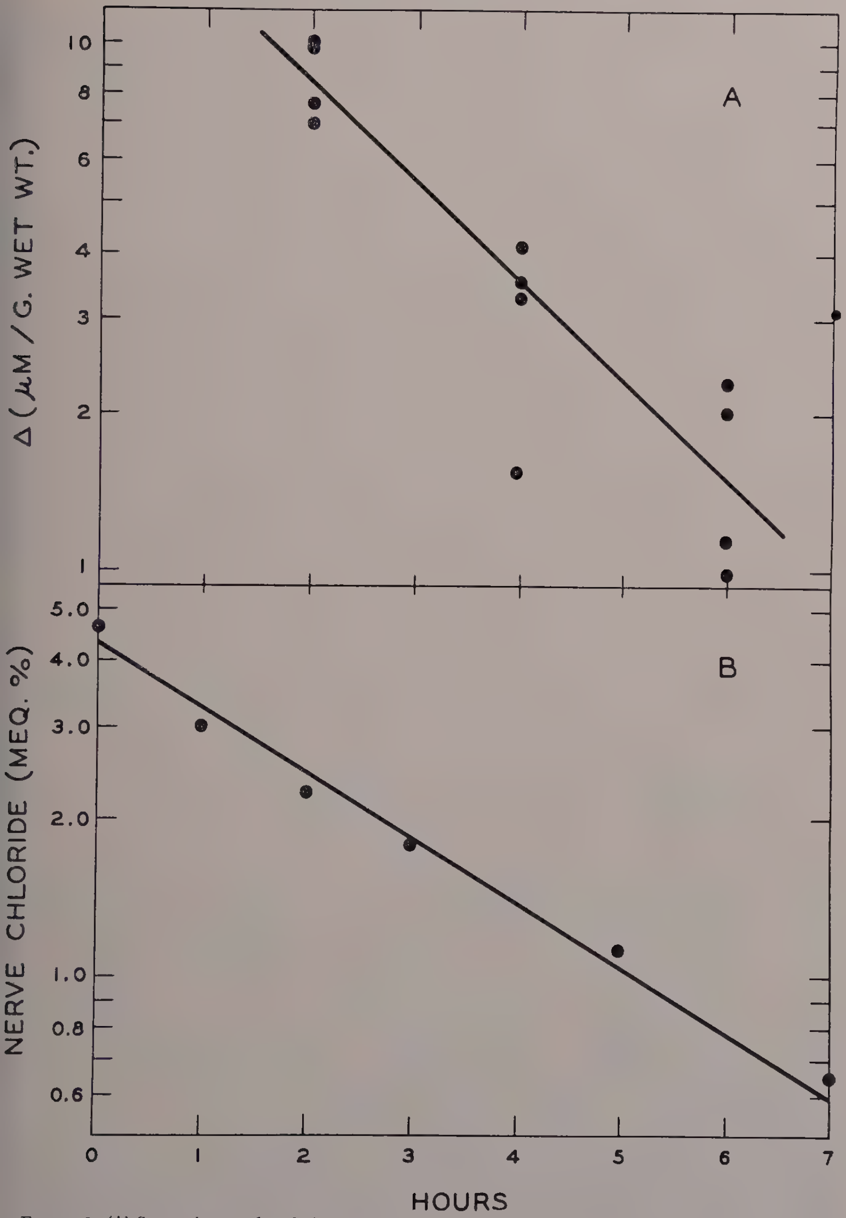

FIGURE 2. (A) Successive two-hourly increments of sodium (Δ) in a 2 mM sodium Ringer in contact with erves equilibrated for one preceding hour in the low sodium Ringer. (B) The decline of nerve chloride in sotonic sugar solution (replotted from Fenn *et al.*, 1934).

Effects of a Low-potassium Ringer. It is known for both vertebrate and nvertebrate nerve that, as the potassium level of the medium is lowered, he resting potential fails to continue increasing as the logarithm of the concentration (Feng and Liu, 1949b; Curtis and Cole, 1942; Shanes and Hopkins, 1948). Indirect evidence based on washing experiments suggests that potassium leakage and accumulation around the fibers is responsible for this (Shanes and Hopkins, 1948). Fenn *et al.* (1934) described a loss of potassium in isotonic sugar solutions. It has been pointed out, however, that substantial potassium losses occur in low sodium even when the potassium content is at Ringer levels. Consequently, the effect of a potassium-free Ringer has been examined.

The results, using 16-hour equilibrated tissue and obtained from nerve analyses for sodium and potassium and from analysis of the medium for potassium, are summarized in FIGURE 3(A and B). The experiments were run in pairs, with one set of nerves in Ringer and the other in Ringer containing 0.1 per cent cocaine. No significant effect by cocaine was apparent from the nerve data, consequently they were combined; each point in FIGURE 3 A being the mean of 16 nerves. The results show a continuous 1:1 exchange of sodium and potassium which is only one-third completed by 24 hours. The media results are in accord with those from nerve analysis; in addition these demonstrate a consistently slower potassium loss in cocaine. This difference is probably significant, for the mean difference of 16 pairs of 3 hour collections was 0.33 μM/g. with a standard error of 0.14 μM/g. Moreover, 12 of the 16 pairs indicated a lower loss in cocaine. The direction of the cocaine effect is in keeping with its action under other conditions.

TABLE 6

POTASSIUM LOSSES TO RINGER, AND SODIUM AND POTASSIUM LOSSES TO A LOW SODIUM RINGER, MEASURED AT SUCCESSIVE INTERVALS, FIRST IN THE ABSENCE, THEN IN THE PRESENCE OF 2 MG. PER CENT VERATRINE (V.)§

Conditions	*Ringer* K *loss*	*Low sodium Ringer**	
		K *loss*	Na *loss*
1 hr. Ringer	0.18 ± 0.21	0.19 ± 0.13	4.58 ± 0.44
1 hr. Ringer	0.0 ± 0.17	0.16 ± 0.09	2.71 ± 0.31
2 hr. V.	3.75 ± 0.49	1.08 ± 0.21	1.65 ± 0.31†
2 hr. V.	5.39 ± 0.44	0.84 ± 0.28	0.70 ± 0.26‡

* NaCl replaced with sucrose or choline chloride.
† The expected sodium loss calculated from oxygen data is 1.96 μM/g.
‡ The expected sodium loss calculated from oxygen data is 0.69 μM/g.
§ Data in μM/g. wet weight; eight paired experiments.

Anoxic Effects. Data obtained in the presence of most of the experimental agents previously studied but over 24 hours are summarized in FIGURES 4 and 5. The differences in the time courses are in general accord with the earlier findings. The release of potassium in the presence of iodoacetate and lactate is identical with that in the inhibitor alone, in keeping with the observation (Shanes and Brown, 1942) that lactate does not influence the acceleration of anoxic depolarization by iodoacetate. The protective action of glucose is striking only over the initial period of anoxia: by 24 hours the potassium loss equals that of the sucrose control. This result, in the light of the irreversible bio-electrical effects described by Lorente de Nó under similar conditions, raises the possibility that prolonged exposure to glucose

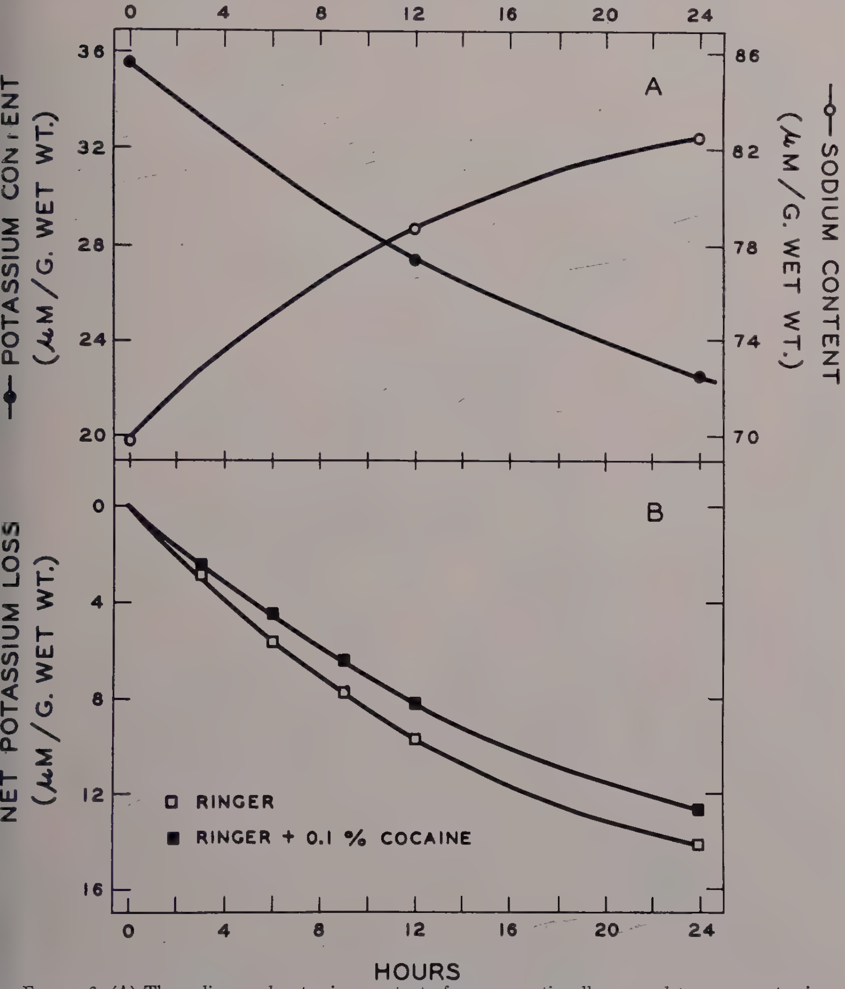

Figure 3. (A) The sodium and potassium content of nerves continually exposed to a zero potassium Ringer. (B) The potassium loss of the same nerves estimated from analyses of the medium in contact with hem.

in the absence of oxygen in some way leads to structural breakdown and simultaneous augmented liberation of potassium. No consistent weight change was obtained over 12 hours of anoxia; by 24 hours, however, weight ncreases averaged about 10 per cent.*

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* Since this represented a volume change of only $\frac{1}{4}$ to $\frac{1}{2}$ per cent for the medium in contact with the nerves luring the second 12 hour period, its effect on the experimental determination of potassium loss during this eriod was negligible.

One question in the evaluation of the anoxia curves was that of the stability of the preparations in oxygen. Observations, therefore, were made on

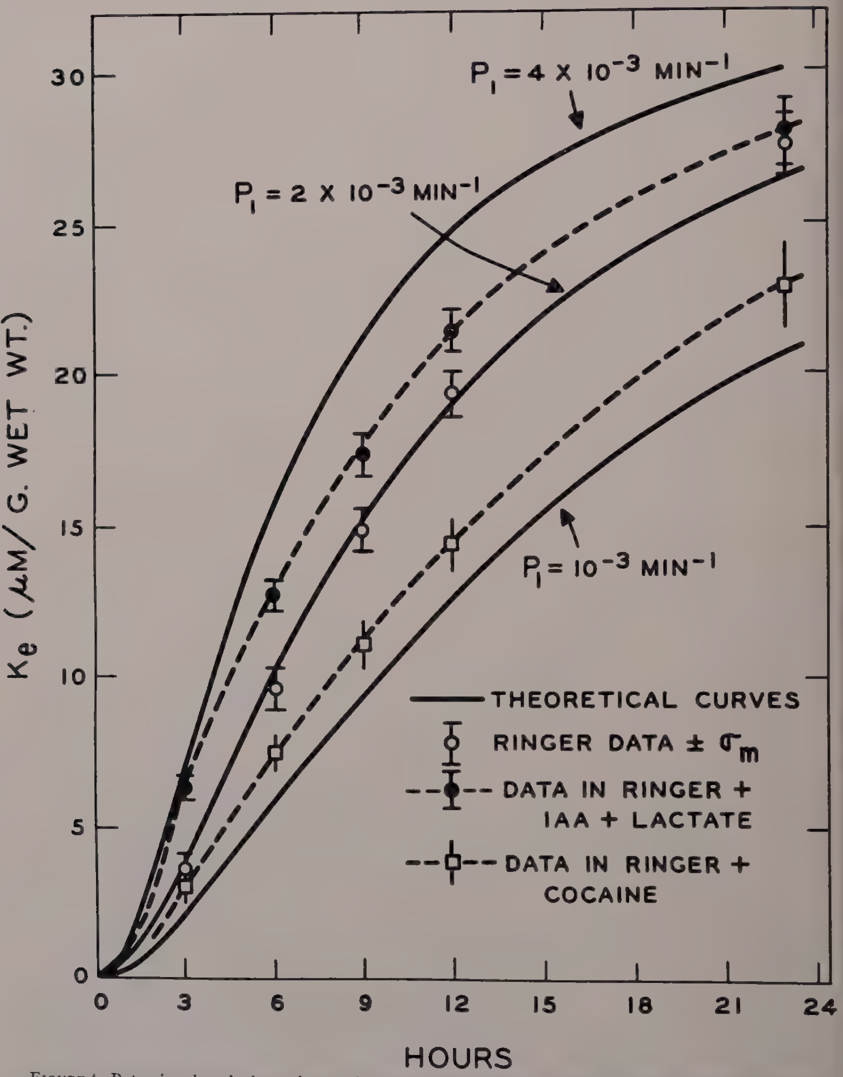

FIGURE 4. Potassium loss during 24 hours of anoxia estimated by analyses of successive samples of medium in contact with nerve.

the sodium and potassium contents of nerves kept in oxygen, and in either regular Ringer or Ringer containing 1 mM iodoacetate with 10 mM lactate. Lactate was added since it reduces the depolarization caused by iodoacetate in oxygen without affecting the action of the glycolytic inhibitor in nitrogen (Shanes and Brown, 1942). The results are given in TABLE 7. It is apparent that the figures in oxygen, referred to wet or dry weight, agree in showing in Ringer a small increase in sodium but no significant change in potassium the first 12 hours, and a negligible change in sodium but a significant decline, of approximately 20 per cent, in potassium by 24 hours. In the glycolytic inhibitor at 12 hours, a 25 per cent decline in potassium is associated with an increase in sodium; by 24 hours, the potassium falls to one-third the original level and the gain in sodium is about equivalent to the loss. Data for nerves paired with the above and kept in nitrogen are given for comparison. The large difference noted between the iodoacetate-treated preparations and the controls in oxygen is considerably less apparent in nitrogen at 12 and particularly at 24 hours.

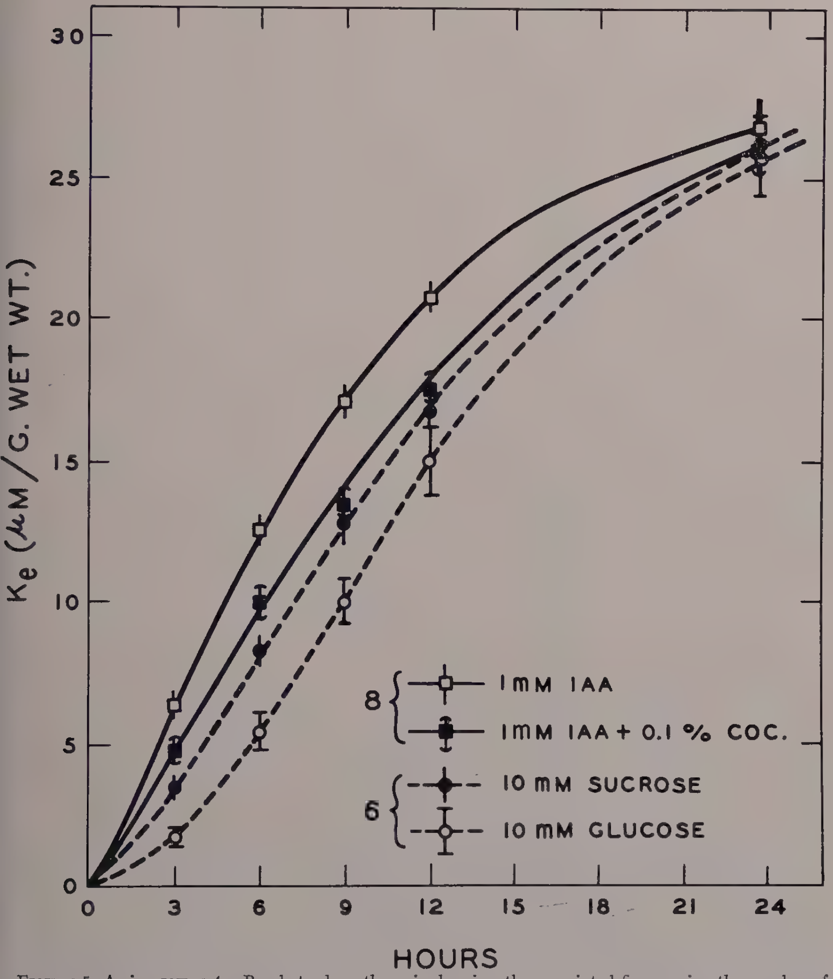

FIGURE 5. As in FIGURE 4. Brackets show the paired series, the associated figures give the number of pairs.

Table 7

Comparison of the Sodium and Potassium Contents of 16-hour-equilibrated Nerves (initial) with Subsequent Values after Additional Periods in Oxygen or Nitrogen in Ringer or Ringer Containing 1 mM Iodoacetate (IAA) and 10 mM Lactate

	Initial				*12 Hours oxygen*				*24 Hours oxygen*			
	μM/g. wet wt.		μM/g. dry wt.		μM/g. wet wt.		μM/g. dry wt.		μM/g. wet wt.		μM/g. dry wt.	
	Na	K	Na	K	Na	K	Na	K	Na	K	Na	K
Ringer	70.1±1.8	33.7±1.7	328±20	156±8	78.5±2.4	33.6±1.8	382±25	162±6.0	73.2±1.2	27.6±1.3	355±9	134±2.6
IAA ± lactate					79.4±2.1	25.1±1.6	381±16	121±6.7	89.1±2.0	11.0±2.3	436±28	52.6±9.3
					12 Hours nitrogen				*24 Hours nitrogen*			
Ringer					91.3±1.5	15.4±1.3	414±10	69.5±5.9	90.9±3.2	7.9±1.4	452±26	38.8±5.3
IAA ± lactate					93.5±1.6	12.0±0.7	436±8	56.1±3.8	95.4±3.3	6.4±1.1	466±33	30.9±5.2

These figures demonstrate a very important fact, *viz.*, that the losses in oxygen cannot be deducted as a correction from those in nitrogen. In the case of the Ringer data, this would lead to a potassium value after 24 hours of anoxia exactly equal to the 12-hour figure. This is totally out of keeping with the continuous escape of potassium observed during the first 12-hour period when the loss in oxygen is negligible. In the case of iodoacetate, such subtraction leads to a smaller net loss after 24 hours than after 12 hours.

A possible explanation for this situation, at least in the case of iodoacetate, follows from its known inhibition of respiration, even when its effectiveness is reduced by lactate (Chang and Gerard, 1933). Thus, if the liberation of potassium in oxygen is due to partial respiratory inhibition, it could not possibly contribute to a greater loss under conditions which completely suppress oxygen consumption. In a more extreme case, *e.g.*, at an inhibitor concentration which completely blocks aerobic reactions, it is obvious that oxygen lack could have no further effect on an oxygen-dependent process such as potassium retention. It is likely that the late loss in oxygenated Ringer likewise is due to depressed respiration and thus does not contribute to additional potassium leakage during the last 12 hours in oxygen-free Ringer. These considerations therefore appear to justify the direct use of the nitrogen data for comparison with theoretical curves.

A corollary of these observations is that the greater effectiveness of anoxia in the presence of iodoacetate need not be ascribed to the leakage initially induced by the inhibitor in oxygen but rather to the inhibition of an anaerobic process, presumably glycolysis, which is partly effective in reducing the escape of potassium. This is in accord with the effect of enhanced glycolysis noted in glucose and of its inhibition by iodoacetate.

A careful comparison of potassium losses calculated from the potassium contents of the nerves and from increments in the medium revealed somewhat larger values for the former. Characteristic results are given in TABLE 8, where this effect is particularly clear at 12 hours. It must be emphasized that the difference is that remaining unaccounted-for after a correction as large as 20 per cent had been added to the media values to allow for (a) the contribution of the extracellular space to the total volume surrounding the fibers, (b) the volume of fluid retained in the perfusion units between collections, and (c) the weight of the short lengths of fine thread used for handling the nerves during the weighings. Incidentally, it may be seen in TABLE 8 that the sodium increment is consistently lower than the potassium loss at these longer times, which differs from the short term effects.

One possible explanation for the larger losses obtained from the nerve data appeared to be that removal of the cut ends left a region of higher initial potassium content, whereas in the media experiments, in which the ends as well as the central segments contributed to the measured potassium losses, the effective initial potassium content was less. The differences obtainable in the first case would therefore be larger than in the second. This was put to the test by comparing the potassium content of the ends of the nerves with that in the central regions. Typical data comparing the proximal and distal ends, averaged together with respect to the immediately adjacent segments and the central-most region, are given in TABLE 9. Both sodium and potassium are uniformly distributed along at least two-thirds the length of the nerve, but show a marked exchange at the ends. From these data, the mean potassium content for the entire length of nerve, taking into account the relative weights of the ends, is found to be 15 per cent less than that indicated by the two central segments alone. This is of the correct order of magnitude to account for the divergence between nerve and media data. Additional analyses have indicated a value between 10 and 15 per cent. The lower is preferable as a correction factor since, in most nerve determinations, less of the ends of the nerves was removed, prior to analysis, than the 1 to 1.5 cm. in TABLE 9.

TABLE 8

COMPARISON OF NERVE AND MEDIA DATA FOLLOWING PROLONGED ANOXIA IN THE PRESENCE OF DIFFERENT EXPERIMENTAL AGENTS. IAA = 1 mM IODOACETATE, Coc. = 0.1 per cent COCAINE*

Hours of anoxia	*Experimental agents*	*Nerve Data*				*Media data*
		Na	Δ Na	K	Δ K	Δ K
0	0 (7)	69.7 ± 2.2	—	35.6 ± 0.8	—	—
12	IAA (8)	92.3 ± 1.1	22.6 ± 2.4	11.1 ± 0.7	24.5 ± 1.0	21.9 ± 0.5
	IAA + Coc. (8)	87.5 ± 1.9	17.8 ± 2.9	13.0 ± 0.8	22.6 ± 1.1	18.6 ± 0.5
	Coc. (8)	82.5 ± 2.0	12.8 ± 3.0	17.1 ± 0.7	18.5 ± 1.0	14.4 ± 0.9
24	IAA (8)	95.1 ± 1.9	25.4 ± 2.9	5.8 ± 0.6	29.8 ± 1.0	28.2 ± 0.6 (4)
	IAA + Coc. (8)	93.6 ± 1.1	23.9 ± 2.4	7.6 ± 0.5	28.0 ± 0.9	27.5 ± 0.8 (4)
	Coc. (4)	88.7 ± 1.8	19.0 ± 2.8	11.3 ± 0.5	24.3 ± 0.9	22.9 ± 1.7 (2)

* Parenthetical figures in column 2 give the number of data for each average in the row except where indicated otherwise.

TABLE 9

COMPARISON OF THE SODIUM AND POTASSIUM CONTENTS OF DIFFERENT PORTIONS OF INDIVIDUAL 16-HOUR-EQUILIBRATED NERVES

Units	*Ends*		*Adjacent segments*		*Central segment*	
	Na	K	Na	K	Na	K
μM/g. wet wt.	94.5	25.7	76.9	40.3	73.6	44.1
	88.0	24.2	76.0	46.8	85.3	46.8
	90.1	27.7	69.1	41.0	70.2	44.8
	96.0	29.7	76.6	47.2	72.8	45.6
Av.	92.2	26.8	74.7	43.8	75.5	45.3
μM/g. dry.wt.	381	103.5	294	155	284	170
	355	97.7	284	175	313	172
	373	115	279	165	264	169
	368	114	238	147	302	189
Av.	369	108	274	161	291	175
Av. wet wt.	31.7		26.5		28.0	

A strict comparison of media and nerve data requires information concerning the relative potassium concentrations in the different regions of the nerve trunk during the course of experimental treatment as well as at the beginning of the experiments. The small magnitude of the deviations involved did not justify an extensive study of this question. Some preliminary data were collected, however, and are given in TABLE 10. These show that, over 6 and 12 hours of anoxia, the potassium at the ends is affected proportionately in about the same way as it is affected centrally. By 24 hours, the potassium level is so low that differences are negligible. The results also indicate, except for the very late times, that the sodium increment at the ends is of the same order as the potassium loss. These observations, in addition to clarifying a source of deviation between media and nerve figures, serve to justify the use of the average nerve concentration (which takes into account the lower potassium content at the ends) in computing the intracellular potassium content and, from it, the theoretical curves of potassium release for comparison with the media data under various experimental conditions.

TABLE 10

COMPARISON OF THE SODIUM AND POTASSIUM CONTENTS, ON A WET WEIGHT BASIS, OF DIFFERENT PORTIONS OF 16-HOUR-EQUILIBRATED NERVES FOLLOWING DIFFERENT PERIODS OF ANOXIA*

Hours in N_2	*Ends*			*Central Segment*			*Mean* K *Conc.*	*Mean* K / *Central* K
	Na	K	*Wet wt.*	Na	K	*Wet Wt.*		
	μM/g.	μM/g.	mg.	μM/g.	μM/g.	mg.	μM/g.	
6(4)	92.2	17.6	60.6	82.7	29.0	45.6	22.5	0.78
12(2)	92.6	7.9	29.0	86.1	13.2	41.5	11.0	0.83
24(2)	108	4.2	31.8	97.8	4.8	50.7	4.6	0.96

* Parenthetical figures give the number of determinations for each average.

Theoretical Considerations

Introduction. The results obtained are in accord, at least qualitatively, with the hypothesis that bio-electrical changes, under the conditions studied, are due primarily to migrations of potassium. That potassium accumulation around the fibers rather than sodium entry causes depolarization and, with it, functional failure, is made clear by two additional types of experiments repeatedly reported in the literature: (1) Metabolically inhibited nerve can be kept functional or may be restored to functioning by mere washing of the fibers in the continued presence of the inhibiting agent (Feng and Gerard, 1930; Feng, 1932; Aykut and Winterstein, 1950; Feng *et al.* 1950a; Shanes, 1951a). Feng *et al.* (1950a) concluded that potassium release and its removal by washing were specifically involved. (2) Anodal currents, which, by virtue of the continued selective properties of the membrane, would drive potassium back into the fibers, restore the polarization level and functional activity under experimental conditions which have now been demonstrated to cause potassium liberation (Lorente de Nó, 1947; Fleckenstein, 1951). Fleckenstein (1951) interprets the anodal effects in terms of the restoration of potassium to the fibers.

With the exception of potassium re-absorption noted upon treatment of veratrinized nerves with cocaine, the results have been restricted to downhill processes. The uptake of potassium during post-anoxic recovery, however, predicted from the potassium hypothesis (Shanes, 1948b; Shanes and Hopkins, 1948), has already been demonstrated (Fenn and Gerschman, 1950; Shanes, 1950a; Shanes, 1951c), as has the uptake associated with repolarization following activity (Shanes, 1951a). It may be assumed, therefore, pending the accumulation of additional data, that potassium uptake represents a reversal of ionic conditions brought about by the downgrade conditions.

The completeness of the antagonism between veratrine and cocaine is suggestive of opposite effects on the same locus in the system. Moreover, the weak effect of cocaine in counteracting metabolic inhibition indicates that veratrine is not acting as an inhibitor. In view of the evidence for a permeability decrease by cocaine and by other "stabilizing" agents (*e.g.*, Höber, 1945), the action of veratrine appears attributable to a permeability increase. Whether this involves a permeability change to sodium, potassium, or both, remains to be determined by more critical methods. Evidence has already been obtained for a specific effect by stabilizers, including cocaine, on potassium permeability (Shanes, 1950b). An increase in sodium permeability would be equivalent to an increase in potassium permeability in so far as potassium transfer is dependent on exchange with sodium. Therefore, no serious error is involved in discussing the action of stabilizers, exemplified by cocaine, and "unstabilizers," exemplified by veratrine (Bennett and Chinburg, 1946; Fleckenstein and Hardt, 1949; Shanes, 1948c, 1951b), in terms of potassium permeability until the problem is further resolved by suitable studies.

The metabolic machinery must be regarded as contributing to potassium retention through a mechanism other than passive permeability. Permeability change, by itself, certainly could not account for ion redistribution with an increase in the free energy of the system, *e.g.*, by potassium reabsorption. As a first approximation, metabolism may be considered to act only by the generation of "forces" [for example, hydrogen ions for exchange (Brooks and Brooks, 1941) or indiffusible intracellular anions (Boyle and Conway, 1941)] which cause the uptake of potassium. The selectivity exhibited by these forces will not concern us here. It may be pointed out, however, that the specificity for potassium could be a consequence of the selective properties (either in terms of pore size or partition coefficients) of the cell surface, as demonstrated by models described by Osterhout *et al.* (1934).

In any case, a separation of metabolic forces from permeability as such simplifies the analysis of the kinetics of ion transfer as well as of bio-electrical changes. Such an analysis is essential for a more critical evaluation of the merits of the proposed hypothesis. This will now be considered.

Kinetics

Potassium Release during Anoxia. In the case of frog nerve, extensive evidence is available for the interference of the connective tissue elements, particularly the external perineurium (or epineurium), with the entry and exit of substances into and out of the nerve trunk (Feng and Gerard, 1930; Feng and Liu, 1949a,b, 1950; Feng *et al.*, 1950b; Crescitelli, 1951; Shanes, 1951c). The long time constant for the diffusion of sodium and chloride, ions which are believed to originate largely in the extracellular space (Fenn *et al.*, 1934), constituted early evidence for this fact.

The simplest description of ion transfer in this tissue therefore requires a consideration of at least two boundaries, that of the individual fibers, which will be assumed to constitute a single layer surrounding a giant fiber, and that chiefly of connective tissue, which will be represented as a lumped extracellular barrier, as shown in FIGURE 6.

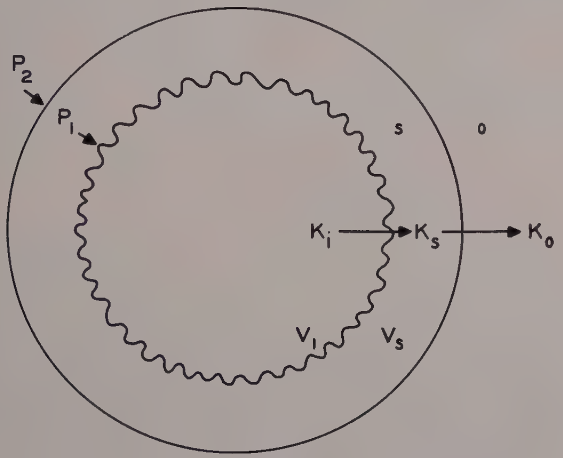

FIGURE 6. Simplified model of a nerve in cross-section.

We may proceed to characterize potassium diffusion during anoxia if the following additional assumptions are made: (1) metabolism can be completely blocked; (2) the permeability of the two barriers in the system remains unaltered during metabolic inhibition; and (3) the activity of potassium, both inside and outside the fibers, is given by the concentration. The second assumption appears justified by the long periods (up to 24 hours, according to Aykut and Winterstein, 1950) that washing experiments have succeeded in preserving functional integrity in the absence of oxygen. We have also noted the beginning return of activity upon restoration of oxygen after the same interval of anoxia. The first assumption seems to be met by combining the action of anoxia with that of iodoacetate, since, according to Feng (1936), lactic acid production accounts for the residual energy release of frog nerve during anoxia. The third assumption satisfies osmotic requirements of the system. The extent to which the relations derived from these assumptions describe nerve behavior and are in accord with thermodynamic considerations will be a further indication of their validity.

When metabolism is turned off, potassium loss from the nerve will occur in two stages; one from the interior of the fibers to the extracellular space, and a second from the extracellular space to the medium. The first stage is described by

$$\frac{dK_i}{dt} = -P_1(K_i - K_s), \tag{1}$$

where K is the potassium concentration, the subscripts i and s indicate that of the intracellular and extracellular space respectively, and P_1 represents the penetration coefficient of the inner boundary. The penetration coefficient is proportional to the permeability, but includes the geometrical factors of the intracellular space, *viz.*, area, A_1, and volume, V_1; thus $P_1 = h_1A_1/V_1$.

The change in K_s with time is given by

$$\frac{dK_s}{dt} = -c\,\frac{dK_i}{dt} - P_2(K_s - K_0), \tag{2}$$

where

$$c = \frac{V_1}{V_s}. \tag{3}$$

V_1 and V_s are the volumes of the intra- and extracellular spaces, the subscript o refers to the external medium where the potassium concentration is maintained constant by frequent changes in solution, and P_2 is the penetration coefficient of the outer boundary.

The simultaneous differential equations are readily solved with differential operators.* The solutions are

$$K_i = K_0 + A_1e^{-(\alpha-\beta)t} + A_2e^{-(\alpha+\beta)t} \tag{4}$$

and

$$K_s = K_0 + 2Ae^{-\alpha t}\sinh \beta t, \tag{5}$$

where

$$\alpha = \frac{P_2}{2}\left[\frac{P_1}{P_2}(1 + c) + 1\right], \tag{6}$$

$$\beta = \frac{P_2}{2}\left\{\left[\frac{P_1}{P_2}(1 + c) + 1\right]^2 - 4\,\frac{P_1}{P_2}\right\}^{\frac{1}{2}}, \tag{7}$$

$$A_1 = \tfrac{1}{2}(K_i' - K_0)\left(1 + \frac{\alpha}{\beta} - \frac{P_1}{\beta}\right), \tag{8}$$

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* The author is indebted to Dr. Jacob Lieberman for pointing out this method.

$$A_2 = \tfrac{1}{2}(K_i' - K_0)\left(1 + \frac{\alpha}{\beta} - \frac{P_1}{\beta}\right), \tag{9}$$

$$A = \frac{c}{P_2} \cdot \frac{A_1(\alpha - \beta)e^{\beta t_m} + A_2(\alpha + \beta)e^{-\beta t_m}}{2 \sinh \beta t_m}, \tag{10}$$

$$t_m = \frac{1}{2\beta} \ln \frac{(\alpha/\beta) + 1}{(\alpha/\beta) - 1}. \tag{11}$$

K_i' is K_i at $t = 0$. K_s passes through a maximum at $t = t_m$, whereas K_i declines in almost exponential fashion.

These equations may now be employed to predict (a) the absolute amount of potassium escaping into the medium (K_e) as a function of time and (b) the bio-electrical changes as a function of time. The former is given by

$$K_e = V_1(K_i' - K_i) - V_s(K_s - K_0), \tag{12}$$

where V_1 and V_s are 0.3 and 0.5 ml./g. wet weight nerve; the latter may be calculated from

$$E = C \log \frac{K_i}{K_s} \tag{13}$$

if the resting potential, E, is assumed to be a membrane concentration difference of potential. The constant C is, in general, considerably less than the usual 58 millivolts by virtue of the divider network set up by the shunting action of the extracellular fluid. An estimate of C is provided by the potential change for a ten-fold increase in extracellular potassium concentration in the linear portion of the E-log K_s plot. This is approximately 20 millivolts in desheathed (Feng and Liu, 1949b) as well as intact (Shanes, 1944) nerve.

Additional constants requiring evaluation are c, P_2, P_1, K_i' and K_0. The last is known, since it is the potassium concentration of the Ringer, *viz.*, 1.7 μM/ml.* K_i' can be determined from the potassium content of whole nerve if the volume of the intracellular space, relative to the extracellular (*i.e.*, c), is known. The chloride data from Fenn *et al.* (1934) indicate a value of $c = 0.6$. The same figure is obtained by a determination of the osmotically active and inactive fractions from the magnitude of the weight change in a Ringer made hypertonic with NaCl (Shanes, 1948d). Allowance must be made, of course, for the relative dry weight (TABLE 3) in the osmotically inactive fraction.

The need to employ a mean K_i' because of variability in the potassium distribution of 16-hour equilibrated nerves has been pointed out. In round numbers, this may be taken as 100 μM/ml.

We come now to an evaluation of the penetration coefficients. Lacking information on the rate of diffusion of potassium through the connective tissue, we will use the data for sodium loss to a low sodium medium to approximate P_2. Since sodium escape is practically an exponential function,

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* The potassium levels of the media in contact with the nerves actually averaged five to ten per cent greater depending on the period of contact; this is neglected in the derivation.

$$P_2 = \frac{\ln (\Delta_1/\Delta_2)}{t_2 - t_1}, \tag{14}$$

which holds when Δ_1 and Δ_2 are the increments in the sodium content of the medium collected over equal periods of time and at successive intervals t_1 and t_2. On this basis, P_2 was calculated from 32 pairs of sodium data, 16 from hourly increments and the remainder from two-hourly increments obtained at later times. No significant difference was found for P_2 calculated in either way. In round numbers, $P_2 = 0.007 \pm 0.002$ min^{-1}, which is in satisfactory agreement with the value of 0.005 min^{-1} obtained from the chloride data of Fenn *et al.* (1934).*

P_1 is now the only term remaining to be determined. Removal of the epineurium causes only a small increase in the rate of potassium liberation during anoxia (Shanes, 1951c). P_1 therefore appears to be the major limiting factor in potassium escape.

An estimate of P_1 is provided by plotting a family of curves for K_e according to equation 12 for different values of P_1 and then comparing experimentally obtained curves with the theoretical ones. This has been done in FIGURE 4. Curves for P_1 from 10^{-3} to 4×10^{-3}, are compared with experimental data obtained in nitrogen with 1 mM iodoacetate or 0.1 per cent cocaine present or absent. It is apparent that the Ringer data coincide closely with the curve $P_1 = 2 \times 10^{-3}$ min^{-1}, that iodoacetate poisoning gives a higher value (*ca.* 3×10^{-3} min^{-1}), and that cocaine treatment lowers the penetrability to about 1.3×10^{-3} min^{-1}. The experimental data conform closely to the theoretical curves in indicating an initial "knee," corresponding to potassium retention in the extracellular space by the connective tissue barrier, as well as in general configuration. Apparently, the experimental curves do not give a clear-cut distinction between the action of metabolic "forces," evident from the improved retention with glycolysis, and of permeability changes brought about by an agent like cocaine; the effects of both are expressible in terms of an equivalent permeability or penetrability. Other more critical procedures, possibly involving the use of radio-active isotopes (*cf.* Ussing, 1949b), appear necessary.

Anoxic Depolarization. The satisfactory description of ion movement achieved with equations 4 and 5 justified an examination of the possibility that they would describe the bio-electrical changes when introduced into equation 13.

The resting potential usually is measured in a moist chamber with the nerve suspended in gas. Under these conditions, diffusion through the second barrier is negligible (*i.e.*, $P_2 = 0$). Lorente de Nó's experiments were conducted with the regions of measurement in contact with a large volume of solution and, therefore, under conditions comparable to those employed in the above calculations. Unfortunately, exact correspondence between the theoretical and experimental curves is unlikely for at least two reasons: (1) Lorente de Nó employed bullfrog nerve, in which the constants are probably different somewhat from those of the leopard frog. (2) The values of both K'_i and K_0 are uncertain, since two different statements are made regarding the extracellular content of potassium: 0.1 per cent (*ca.* 13 μM/ml.) and 1.3 μM/ml. (Lorente de Nó, 1947, Part 1, pp. 17, 32). Although the lower value is probably the one used, it is so low that potassium leakage must have occurred continuously in these nerves. FIGURE 7 is a plot, similar to that employed by Lorente de Nó, of demarcation potential change* in nitrogen against the logarithm of time calculated from equation 13, using the constants which fit the Ringer data for *R.* pipiens. An initial "slow phase" is followed by a linear decline which, in turn, is succeeded by a "slow phase." The general features of the curve, as well as the approximate times of the three stages, are essentially as described by Lorente de Nó (1947, Part 1, pp. 46, 47). The amplitude of the potential change is approximately double that of his experimental figures.

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* It should be noted that this value for P_2 must be regarded as a rough estimate; it has been measured using not only ions other than potassium but freshly dissected nerves, rather than the 16-hour preparations actually employed for comparison with theory.

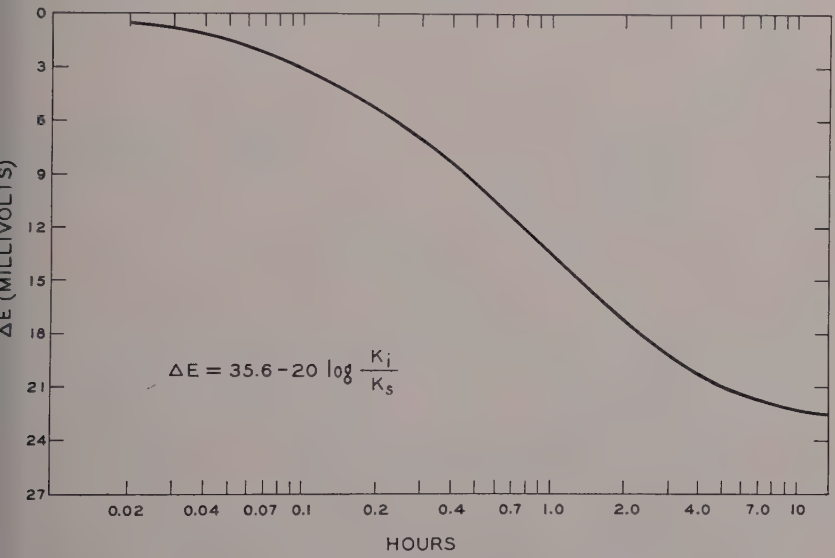

FIGURE 7. Theoretical time course of demarcation potential development in nitrogen.

A closer comparison with available experimental bio-electrical data appeared possible by recalculation of K_i and K_s for the situation of nerves mounted in gas. In this case, only P_1 need be considered, so that equation holds, but

$$K_s = K_0 + c(K'_i - K_i). \tag{15}$$

Therefore,

$$K_i = \frac{K_0 + cK'_i}{1 + c} + \left(K'_i - \frac{K_0 + cK'_i}{1 + c}\right) e^{-P_1(1+c)t} \tag{16}$$

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* Change in potential of a sigment of nerve in nitrogen relative to another intact segment in oxygen.

Data were obtained by Shanes and Brown (1942) under conditions approaching those assumed in the derivation of equation 16. A narrow strip of filter paper moistened with Ringer made contact with the region of nerve subjected to nitrogen. Consequently, although some potassium diffusion was possible, it must have occurred to a lesser degree than in Lorente de Nó's experiments. In FIGURE 8, data so obtained (taken from the earlier experiments) are compared with the theoretical curve based on the constants for Ringer-treated nerve. The results are consistent in showing a decline slower than the theoretical one at later times. Initially, the iodoacetate preparations approximate the theoretical curve, but the Ringer nerves show a definite deviation from the very beginning, suggesting a smaller P_1 value than estimated from FIGURE 4. This deviation from theory requires further study. Bio-electrical measurements must be carried out under more carefully controlled conditions, but it is particularly important to ascertain whether the penetrability and other constants, obtained for the whole nerve with its varying dimensions and based on sodium and chloride diffusion rates, are applicable to the bio-electrical measurements, where only short segments of nerve were involved.*

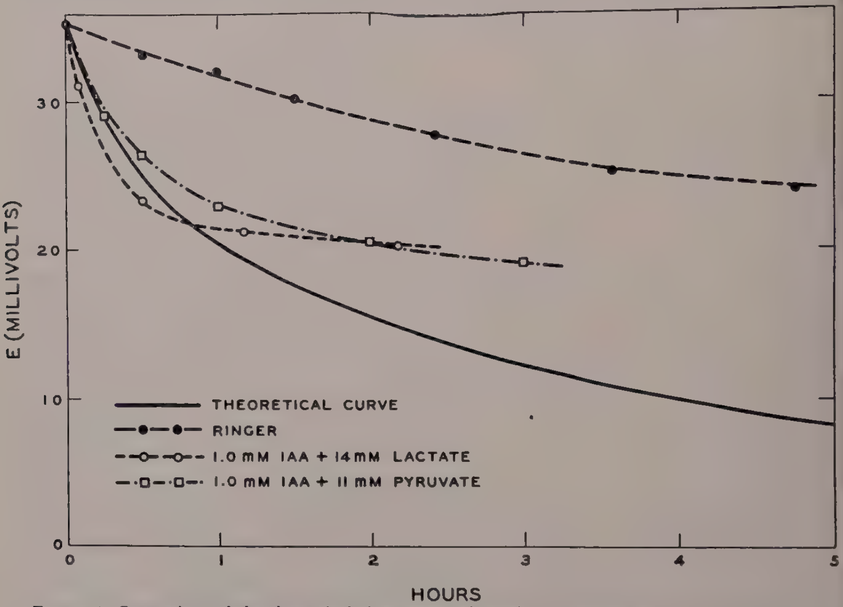

FIGURE 8. Comparison of the theoretical time course of anoxic depolarization with the published and unpublished data from Shanes and Brown (1942).

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* It is important to note in this connection that the membrane concentration potential employed in these calculations has taken into account only the potassium ion. Since it is now evident that the sodium ion also can diffuse (although the membrane permeability to it is considerably less than that of potassium, as indicated by its relatively weak electrical effects) and since the anions do not contribute appreciably to ionic transfer (*i.e.*, anion permeability zero), one may apply either the Henderson or Planck diffusion equation with the following result:

$$E = C \log \frac{bNa_i + K_i}{bNa_0 + K_0}.$$

The coefficient b is the ratio of sodium to potassium peremability; it remains to be evaluated, but is probably

Potassium Release in Low Potassium Solutions. The time relations established for potassium escape in the absence of medium potassium provide a further test of the theoretical approach which is being applied. Metabolic processes must now be considered together with the more passive elements of the system.

The respiratory rate of nerve (and muscle) has been found, within certain limits, to be directly related to the external potassium concentration (see Shanes, 1949a for references). As a first approximation, we shall assume a simple linear relationship. In view of the evidence (Shanes and Hopkins 1948), a corresponding relation may be assumed to exist between the forces of potassium uptake and the potassium concentration at the surface. Therefore,

$$\frac{dK_i}{dt} = kK_s - P_1(K_i - K_s), \tag{17}$$

where k is the rate constant of the metabolic mechanism and the other terms are the same as in equation 1. In the steady state $\frac{dKi}{dt} = 0$, and since $K_i \gg K_s$,

$$\frac{k}{P_1} = \frac{K'_i}{K'_s} = \frac{K'_i}{K_0}. \tag{18}$$

The primed terms represent steady state values. Thus, the ratio of the steady intracellular concentration to the extracellular concentration defines the magnitude of k relative to P_1.

For the case $K_0 = 0$, equation 2 becomes

$$-\frac{dK_s}{dt} = c\,\frac{dK_i}{dt} + P_2K_s. \tag{19}$$

The solution of equations 17 and 19 is of the same general form as equation 4:

$$\frac{K_i}{K'_i} = B_1e^{-(\gamma-\delta)t} + B_2e^{-(\gamma+\delta)t}, \tag{20}$$

in which

$$\gamma = \frac{P_2}{2}\left[\frac{P_1}{P_2}(1+c) + \frac{k}{P_2}c + 1\right], \tag{21}$$

$$\delta = \frac{P_2}{2}\left\{\left[\frac{P_1}{P_2}(1+c) + \frac{k}{P_2}c + 1\right]^2 - 4\frac{P_1}{P_2}\right\}^{\frac{1}{2}}, \tag{22}$$

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o more than 0.01. Such a value for b will indeed reduce the calculated potential changes by the order of nagnitude required.

Until b is experimentally determined, as may be possible with radioactive isotopes, the actual application f this equation in calculations such as given above would be premature. It is worthy of note, however, hat A. Lundberg (1951 Acta Physiol. Scand. 22: 365) finds that sodium excess depresses depolarization by otassium to a greater extent than other "indifferent" ions which serve to correct for the greater shunting nder these conditions. Unfortunately, quantitative data are not presented, but the direction of the effect s that indicated by the above equation. The same equation demonstrates that the replacement of extra-ellular potassium (K_o) with another ion of nearly equal penetrability (*e.g.*, rubidium), will lead to an equal lepolarization. Consequently, the objection to a membrane concentration potential on the basis of the lepolarizing action of *Rb* in the absence of any actual Rb_i/Rb_o ratio (*e.g.*, Feng and Liu, 1949) is not valid.

$$B_1 = \frac{1}{2}\left[1 + \frac{P_1}{\delta}\frac{K_s'}{K_i'} + \frac{\gamma}{\delta} - \frac{P_1}{\delta}\right], \tag{23}$$

$$B_2 = \frac{1}{2}\left[1 - \frac{P_1}{\delta}\frac{K_s'}{K_i'} - \frac{\gamma}{\delta} + \frac{P_1}{\delta}\right]. \tag{24}$$

The value of k/P_1 under the conditions actually employed is 60. Since $P_1 = 3 \times 10^{-3}$ min^{-1} (from the IAA curve in FIGURE 4) and $P_2 = 7 \times 10^{-3}$ min^{-1}, we have $k/P_2 = 26$. Solutions for this case and for P_1 or k reduced by $\frac{1}{3}$ are compared with the actual nerve data in TABLE 11. The experimental figures show a greater potassium release than predicted for normal nerve. They are closely approximated, however, by a curve for a metabolic rate two-thirds normal. This is of interest in view of the metabolic depression suggested by the liberation of potassium during the 12- to 24-hour period by nerves in regular Ringer (TABLE 7). Aside from any theoretical considerations, the loss in such controls is large enough to account for the discrepancy between the theoretical and experimental values at 24 hours.

TABLE 11

COMPARISON OF NERVE POTASSIUM CONTENTS (μM/g. WET WEIGHT), AFTER DIFFERENT PERIODS IN ZERO POTASSIUM RINGER, WITH THEORETICAL VALUES DERIVED USING PREVIOUSLY EVALUATED CONSTANTS, EXCEPT AS INDICATED

Time (hours)	*Experimental*	$k/P_1 = K_i'/K_s'$ $P_1 = 3 \times 10^{-3}\text{min}^{-1}$	$k/P_1 = K_i'/K_s'$ $P_1 = 2 \times 10^{-3}\text{min}^{-1}$	$k/P_1 = 2K_i'/3K_s'$ $P_1 = 3 \times 10^{-3}\text{min}^{1}$
0	35.7 ± 0.8	35.7	35.7	35.7
13	27.4 ± 1.2	30.3	32.0	28.1
25	22.5 ± 0.9	26.6	29.4	23.0

Nevertheless, it would be desirable to follow the respiratory rate in zero potassium experiments since this would provide a measure of the deviation of metabolism from the simple relationship which has been assumed.

A reduction in P_1, such as would occur in cocaine, slows the theoretical rate of potassium release. It has already been pointed out the data indicate an effect in the expected direction. The magnitude of the difference to be expected for three-hour collections over the first twelve hours is approximately one fourth the theoretical difference found at thirteen hours between $P_1 = 2 \times 10^{-3}$ and $P_1 = 3 \times 10^{-3}$, *viz.*, 0.4 μM/g. This is in satisfactory agreement with the figure 0.33 ± 0.14 found experimentally.

Concluding Remarks

General. The available data have shown (1) a perfect qualitative correlation between the direction or alterations in the rate of potassium movement and the behavior of the resting potential under a variety of conditions and (2) a satisfactory agreement with a kinetic analysis of potassium leakage during anoxia and in low potassium based on a separation of physical restraints to diffusion from an active metabolic mechanism. They have also established a close relation between potassium release and sodium uptake. Although the former can occur independently, it is dependent on the latter under certain conditions.

The theoretical approach employed does not provide an unequivocal distinction between restraints to potassium escape of a purely physical nature and those set up by metabolism. It is possible, however, that the active aspect of the latter may prove distinguishable from the more passive permeability effects in studies of the exchange rates of radioactive ions.* The use of radioactive isotopes would be desirable as a further check on the penetration coefficients, particularly P_1, as evaluated by the present kinetic analysis.

A discrepancy in the absolute magnitude of the theoretically calculated and actual bio-electrical changes was noted. Technical procedures were sufficiently different in earlier bio-electrical studies to justify their repetition under conditions more comparable to those assumed in the theory; a check on P_1 and P_2 values also appears desirable in this connection.

Agreement with theory nevertheless appears sufficient to suggest that some of the major general characteristics of the system, with respect to potassium transfer, are defined. More subtle aspects, such as the distinction between potassium and sodium permeability, or between permeability and metabolic "forces," remain to be resolved by more suitable techniques.

Energetics. A further test of the original assumptions is provided by energy considerations. Thus, with the metabolic machinery completely cut off and the permeability characteristics unchanged, the rate of potassium escape equals the original rate of uptake in the steady state. From this, the rate of work under resting conditions may be calculated for comparison with energy turnover data available from other measurements (Shanes, 1951 c). The efficiency of about ten per cent so obtained is of a reasonable order of magnitude and in keeping with that computed from bio-electrical measurements in other systems (Crane, 1950). On this basis, then, the postulate that permeability during inhibition is essentially that during steady state conditions appears to be acceptable. A substantial increase in permeability, which is the more likely direction of any change, would have led to excessively high efficiency values.

The adequacy of Hydrogen Ion Exchange. One of the mechanisms mentioned as a basis for potassium uptake and retention is that of exchange with metabolically generated hydrogen ions (*e.g.*, Brooks and Brooks, 1941). Evidence is available for such exchange in other systems (Conway and O'Malley, 1945; Rothstein and Enns, 1946) as well as in frog nerve (Shanes, 1948 b).

The rate of hydrogen ion production required is indicated by the maximal potassium leakage rate as given by equation 1. This is of the order of 5 μM/g. hr. for $P_1 = 3 \times 10^{-3}$ min^{-1}, $K_i' = 100$ μM/ml., and $V_1 = 0.3$ ml/g. Oxygen consumption values range from 16 to 56 mm^3/g. hr., the higher limit being favored by thermal measurements (Feng, 1936). 50 mm^3 is equivalent to 2.1 μM of O_2, or the production of 2 μM of CO_2. Consequently, even complete dissociation of H_2CO_3 could not supply hydrogen ions at the rate required to balance the potassium outflux based on the estimated value for P_1. Unless P_1 is seriously in error, it appears likely that another mechanism contributes, at least in part, to potassium retention.

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* Thus, it may be anticipated that ion exchange would be enhanced by augmented active transport or epressed by reduced peremability, depending on the predominant cause of improved potassium retention.

As already pointed out, the actions of glucose and iodoacetate under anaerobic conditions are consistent with their effects on lactic acid and, therefore, on hydrogen ion generation (Shanes, 1948b). The rate of acid production has been reported to be between 2 and 3 μM/g. hr. at room temperatures (Schmitt and Cori, 1933; Gerard and Meyerhof, 1927), which is about the difference in potassium loss in Ringer and IAA treated nerves. Glucose almost doubles the initial rate of acid production (Gerard and Meyerhof, 1927), which again is large enough for the improved potassium retention in this sugar. The order of magnitude of hydrogen ion production during anoxia is therefore in better agreement with the amounts of potassium retained than that computed from CO_2 production under aerobic conditions.

Implications for Transient Properties of Nerve. The dependence of slow bio-electrical changes on potassium raises the question of whether this may also be true for the more labile characteristics of nerve. That such may be the case appears indicated by the predictability of potassium shifts associated with negative and positive after-potentials (Shanes, 1951a) and by the antagonism between veratrine and cocaine with respect to subthreshold as well as threshold phenomena (Shanes, 1949a).

Recently, certain experimental conditions—, e.g., the stabilization by anaesthetic agents like cocaine (Bennett and Chinburg, 1946) or the depression of spike height by lowered sodium levels (Hodgkin and Katz, 1949)—have been assumed to have a specific action on the spike, because of the absence or the weakness of an effect on the steady level of the resting potential. It was pointed out some time ago, however, that an alteration in the properties of the resting potential may be demonstrable only when the potential fluctuates. Under these circumstances, the rate of change, or the amplitude of change per unit time, is found to be modified (Shanes, 1948a, b, c, 1949a and b). More properly, therefore, the validity of such specificity should be determined by a comparison of the effects on another type of change in potential (e.g., a change induced by inhibition or by veratrine). In the case of veratrine depolarization (and potassium release), cocaine causes complete block at concentrations which block spike production. Now, low sodium in the medium also has been found to curtail drastically the release of potassium by veratrine, from which it may be guessed that a similar blocking effect will be found for veratrine depolarization. Such action by low sodium on other types of depolarization (anoxia, negative after-potential, and oscillations) has been observed already but will be described in detail elsewhere. It will suffice here to point out that evidence now exists that effects, previously ascribed to special properties of the spike, in particular those induced by stabilizing anaesthetics or low sodium, have their counterpart with respect to other fluctuations in polarization level. Veratrine depolarization is of special interest, since its sensitivity, being comparable to that of the spike, is suggestive of the same fundamental mechanism.

On such grounds and, on the basis of the evidence which has been presented, that a potassium rather than a sodium shift is the most immediate cause of bio-electrical change, one might conclude that spike production represents an exchange of sodium for potassium, the rapid efflux of the latter causing a transient reversed potassium gradient and, thereby, the now well-known reversal of polarization. A reduction in the sodium content of the medium, by reducing the ionic exchange, would restrict the potassium shift and with it the extent of polarization reversal; the dependence on sodium would be logarithmic insofar as the polarization still varied as the ogarithm of the potassium ratios.

The recent report on inward and outward currents by Hodgkin *et al.* (1949) is difficult to assess because of its brevity. However, the inward current and its alterations with sodium level observed when the membrane potential drop was maintained constant by feedback appears explicable, at least qualitatively, in terms of ionic exchange with a potassium-induced depolarization. In this case, the feedback circuit would itself be the source of inward current since it would be neutralizing an E.M.F. change which had occurred without the one-way cation transfer which Hodgkin and his associates assume. Similarly, the outward current need not signify an exit of cations.

The problem of elucidating the sequence of events associated with spike production is a formidable one. The "frontal assault" by the English nvestigators has led to many important facts. Their interpretation, however, hinges on the validity of the assumptions involved in the methods. The studies described in the present report constitute an attack from the rear in so far as agents of importance in the transient phenomena can be exhibited to exert similar effects on slower processes more directly accessible to other than electrical measurements. These agree in assigning an important role to sodium, but suggest that this ion is not responsible for depolarization but rather is needed to permit the rapid exit of potassium, which actually causes the bio-electrical changes. In this connection, the role of he myelin and Schwann sheaths certainly requires evaluation. Their presence renders questionable the assumption that fiber capacitance is ocalized in the "membrane" or that diffusion beyond the axon surface is ompletely free. The possibility of accounting for the oscillatory electrical properties of the axon when the sheaths and lagging processes are considered has been pointed out (Shanes, 1949a). The erroneous conclusions which previously resulted from a disregard of the more obvious epineurium* cerainly dictate caution with respect to the less accessible sheathing elements f individual fibers.

Summary

Experimental Results. Oxygen lack, iodoacetate poisoning, low potasium in the medium, and depolarizing concentrations of veratrine cause a continuous release of potassium and the uptake of roughly equivalent amounts of sodium in frog nerve. Depending on experimental conditions, sodium entry exceeds or is less than potassium loss; in low sodium media potassium escapes without exchange.

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* Additional observations to those already quoted are provided by Rosenblueth and Luco (1950) and ashbass and Rushton (1949).

Cocaine depresses ionic interchange under all conditions, but is most effective against veratrinization. Low sodium media are also more effective against the potassium escape induced by veratrine than by anoxia. Glucose reduces the ionic transfers of anoxia, an effect which is counteracted by iodoacetate; low concentrations of iodoacetate and of veratrine have the opposite effect.

Theoretical Results. The data are qualitatively in accord with the earlier hypothesis that bio-electrical changes occurring under these conditions are due to potassium liberation and accumulation around the fibers. The experimental evidence indicates that the sodium in the medium may contribute to potassium escape by exchange and possibly by maintaining a higher permeability, thereby affecting the electrical phenomena indirectly.

On this basis, a quantitative theory is developed for the kinetics of potassium loss under conditions of anoxia and of low potassium in the medium. Fundamental postulates include (1) the separation of metabolic "forces" for potassium uptake from the passive physical factors leading to potassium escape and (2) consideration of two barriers to diffusion, one of the extracellular connective tissue and the other the fiber membranes themselves, both of which retain their permeability characteristics during metabolic inhibition.

Within the limitations of the data, the derived relationships are found to describe potassium movement over 24 hours. The time course of bioelectrical change during anoxia, computed on the assumption of a potassium membrane concentration potential, conforms with that described under corresponding experimental conditions for another species of nerve. The calculated amplitude of the electrical shifts is too large, but whether this is due to the different experimental material or to other factors remains to be determined. Comparison of theoretical and experimental curves obtained for ordinary moist chamber conditions likewise shows smaller depolarizations than predicted. The source of this discrepancy also remains to be determined.

The calculated efficiency with which the steady state conditions are maintained is about ten per cent, which is in reasonable agreement with other systems. Hydrogen ion generation during anoxia is adequate to account for potassium retention, but that from CO_2 production under aerobic conditions is found to be insufficient.

Conclusions. The directions of the slow bio-electrical effects of metabolic agents and "stabilizers," exemplified by cocaine, and "unstabilizers," exemplified by veratrine, now appear accounted for in terms of the directly determined alterations in potassium retention. Although final proof hinges on the application of other available methods, the evidence points to two distinct mechanisms by which potassium retention is altered: by modifications in the intensity of metabolic "forces," effected by metabolic agents, nd by changes in permeability, effected by stabilizers and unstabilizers. Vhether the permeability change is to sodium, potassium, or both, also equires elucidation.

The similarity of the interactions on a slow time scale with those known or the much faster subthreshold and threshold phenomena of nerve offers n important basis for the elucidation of mechanisms less susceptible to irect methods.

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